Poisonous Animals and Their Venoms

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The etymologists tell us that the word for *venom* is derived from the Latin *venenum, i.e.*, drug, poison, magic charm (related to *venus,* love), and use of this word should be restricted to poisonous matter secreted by animals, such as snakes, scorpions, and bees. While *venom* denotes origin, *poison* refers to effect and includes any substance that on entering living organisms in small quantities has harmful or fatal properties. The term *toxin,** as we use it nowadays, refers to poisonous proteins elaborated during metabolism of living organisms, especially of bacteria. As a defense against toxins, living organisms prepare *antitoxins.* Venoms are sometimes referred to as *biotoxins,* a term which should be reserved for *proteinaceous* venoms (1). Some of these active agents are listed in Table I, which gives approximate minimal lethal doses per microgram (0.000001 g.) of compound for a few representatives from plants and animals (2-7).

While the cobra *(Crotalus terrificus)* makes *active* use of its neurotoxin, the puffer fish *(Spheroides rubripes),* the Colombian poison arrow frog *(Phyllobates bicolor),* toad, and salamander contain *passive* venoms which act only when these animals are eaten or their extracts enter the blood stream. Interestingly enough, salamanders will die of their own venom when, as a result of some lesion, it penetrates from the skin glands into the blood.

Since antiquity the ingredients of plants and animals have been used as arrow poisons for hunting. In Guam the natives poison the pools among the coral reefs with the juices pressed from sea cucumber *(Holothuria argus)* as an aid in catching fish for food. The active principles in the Bahamian sea cucumber *(Actinopyga agassizi)* are concentrated in the Cuvierian tubules, which are reddish, branching filaments containing granules and which are attached to the common stem of the respiratory organs near the region where the intestinal tract enters the cloaca. When the sea cucumber is disturbed, it may react by a vigorous contraction of the body wall, followed by a slow extension of the Cuvierian tubules through a rupture in the cloacal wall, and finally by an explosive expulsion of the intestinal tract and genital glands out through the anus. Autotomy occurs when these organs break off from the rest of the body. The structures remaining within the animal are remnants of mesenteric tissue, the cloaca respiratory organs, the anterior tentacles, and all parts of the water vascular system. As time progresses, the eviscerated and autotomized parts are regenerated(8).

The exact relationship of the poison-laden Cuvierian tubules to the phenomenon of evisceration is not definitely known, although the available facts intimate a close association. Injection of holothurin solutions made from fresh tubules will induce evisceration as will the introduction

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* *Taxus* = yew, probably furnished wood for *toxon* = bow, which gave rise to *toxicon* (pharmakon) = (arrow) poison; the Greek word for arrow is *ia,* which in *iatros* = *physician,* German: Arzt, entered into the therapeutic application of poisons in medicine.

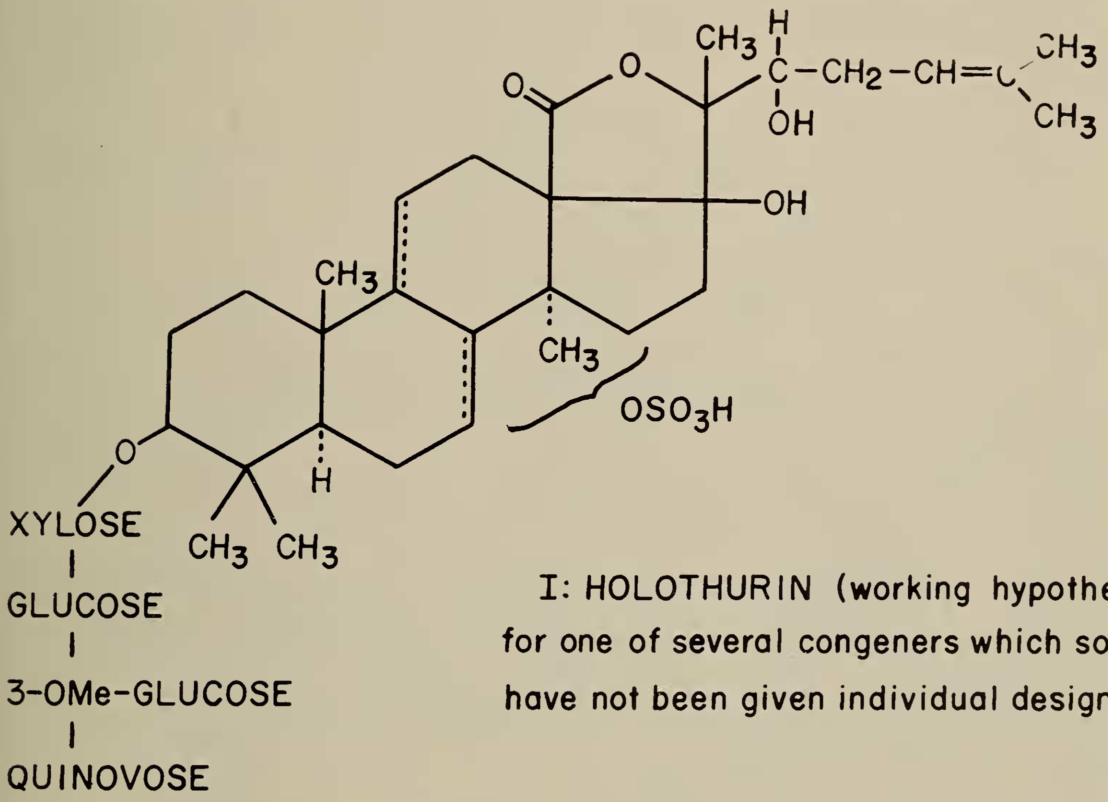
Table I. *Toxicity of the Most Active Naturally-occurring Poisons*

Venom, toxin, or poison	Class of compound	Toxicity MLD/μg of cpd.	Animal	Reference
Botulinus toxin, crystalline type A	Protein, MW 900,000-1,130,000	1,200 30,000	Guinea-pig Mouse	C. Lamanna et al., Science **103**, 613 (1946).
Tetanus toxin	Protein, MW 67,000	1,200 12,000	Guinea-pig Mouse	W. van Heyningen, *Bacterial Toxins* (Thomas, Ill. 1950), p. 6.
Diphtheria toxin	Protein, MW 72,000	3.5	Guinea-pig	
Batrachotoxin from the Colombian poison arrow frog, *Phyllobates bicolor*	Nitrogenous steroid, MW 399 (*cf.* X).	50-100	Mouse	F. Märki and B. Witkop, Experientia **19**, 329 (1963); J. Daly, B. Witkop, P. Bommer and K. Biemann, J. Am. Chem. Soc. **87**, 124 (1965).
Calabash curare alkaloid E Calabash curare alkaloid G	Dimeric indole alkaloids related to strychinine	0.95-8 0.7-12	Mouse Mouse	J. Kebrle, H. Schmid, P. Waser, and P. Karrer, Helv. chim. Acta **36**, 116 (1953).
Paralytic shell fish poison (mytilotoxin)	Guanidine derivative, MW 372	5-6	Mouse	E. J. Schantz et al., J. Am. Chem. Soc. **79**, 5230 (1957).
Tarichatoxin (eggs of California newt)	Identical guanidine derivatives (II, III, IV)	7	Mouse	M. S. Brown and H. S. Mosher, Science **140**, 295 (1963); **144**, 1100 (1964).
Tetrodotoxin (poison from toxic puffer or globe fish)	$C_{11}H_{17}N_3O_8$, MW 319	3-5	Mouse	E. F. Murta, Ann. N.Y. Acad. Sci. **90**, 821 (1960).
Gonyaulax catenella poison (purif.)		5	Mouse	E. J. Schantz et al., Am. Chem. Soc. Meeting (Sept. 1962).
Coral poison ("palytoxin")	Weak base, MW 650 (?)	2	Mouse	P. Scheuer, Univ. of Hawaii (unpublished)
Samandarine from fire salamander (*Salamandra maculosa*)	Modified nitrogenous steroid, MW 305, $C_{19}H_{31}NO_2$ (VI)		Mouse	O. Gessner and P. Möllenhoff, Arch. exptl. Pathol. Pharmakol. **167**, 638 (1932).
Cobra venom neurotoxin	Protein, MW 30,000	0.9	Mouse	*Venoms*, edited by E. E. Buckley and N. Porges, Amer. Assoc. Adv. of Science (Washington, D. C., 1956).
Sea cucumber venom (holothurin)	Steroidal sapogenin, glycoside, MW-1200 (Structure I)	0.1	Mouse	S. L. Friess et al., N. Y. Acad. Sci. **90**, 893 (1960).

of this solution or of the tubules into the water in which intact animals are kept. The higher the dose of the venom, the quicker is the reaction.

Crude holothurin has cancerostatic properties. Even 0.1 mg. of such a preparation, injected intraperitoneally into ascites-bearing mice, leads to a remarkable increase in survival time.

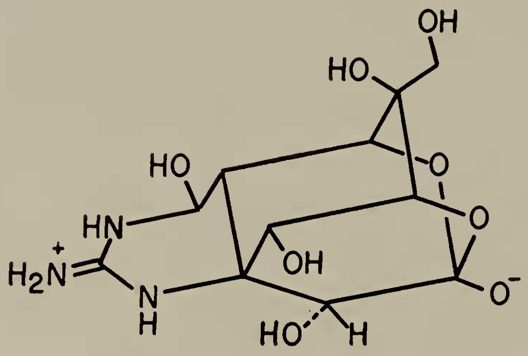
Holothurin consists of two fractions: the A-fraction resembles the plant sapogenin digitonin, and forms an insoluble complex with cholesterol. The aglycon is the sulfuric ester of a steroid lactone I to which is attached the following sequence of four monosaccharides: quinovosyl (3-0-methyl-glucosyl)-glucosylxylose. This is the first instance of the isolation of a steroidal sapogenin from animals (9). Only plants have been known to contain this class of compounds. Even more exceptional is the triterpenoid sapogenin which recently was reported to occur in *Holothuria vagabunda* (10).



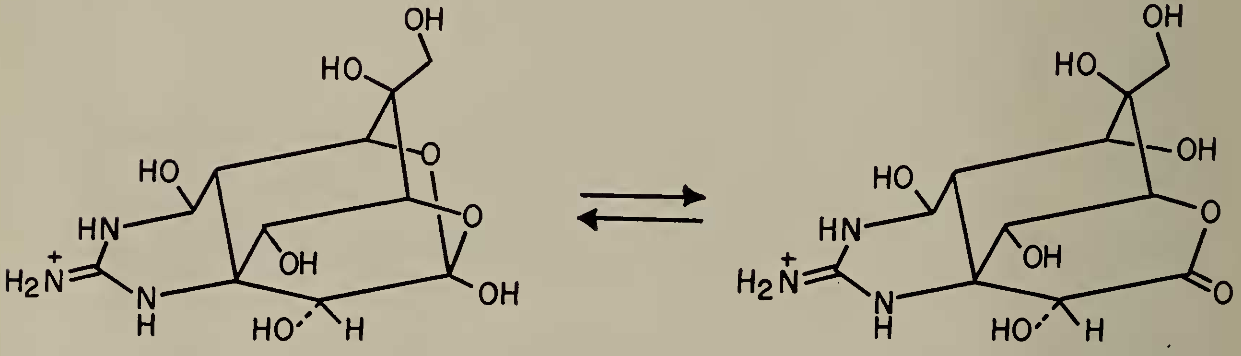
I: HOLOTHURIN (working hypothesis for one of several congeners which so far have not been given individual designations).

Whereas utilitarian principles led to the discovery of venoms and arrow poisons for hunting purposes, gourmandism detected the most dreaded marine venom. Nearly 500 persons died in Japan during 1956–1958 as a result of poisoning from eating *shashimi* (raw portions) of *fugu, i.e.*, puffer fish *(Spheroides rubripes* and *porphyreus). Ichthyosarcotoxism* is the high-sounding term for this syndrome which also punishes eaters of other fish, such as certain morays *(Gymnothorax)*, mackerels *(Scombroidei)*, and *Ciguatera*. Only licensed operators in Japan are allowed to serve the dangerous delicacy to gourmet customers. The venom is localized in the livers and ovaries of the puffer fish, whose excision is mandatory for the purpose of consumption. The venom was isolated, crystallized, and named tetrodotoxin in 1950. Tsuda determined its toxicity as 0.01 γ/g. in mice. It required the most modern methods for two Japanese teams (Tsuda and Hirata) and one group at Harvard (R. B. Woodward) to arrive at the correct empirical formula and three-dimensional structure of textrodotoxin. The difficulty of this elucidation is easily seen from the formula, $C_{11}H_{17}N_3O_8$, in which the number of hetero-atoms matches the number of carbon atoms, nine of which are asymmetric. The free tetrodotoxin base is a zwitterion II which on protonation becomes the hemilactal III, which is in equilibrium with the hydroxylactone IV (11).

The dimeric ether structure V, a serious alternative suggestion for I, could only be ruled out on the basis of a careful determination of the unit cell and the molecular weight of tetrodotoxin by X-ray crystallography (12).



II. Tetrodotoxin Zwitterion (free base)



III. Hemilactal (salt)

IV. Hydroxylactone (salt)

If we now turn our attention from marine to amphibian venoms, we notice some interesting relationships. The classical work in this area begins with the toad venoms (H. Weiland, 1920-1943 (13)), continues with the salamander (C. Schöpf, 1930-1961 (14)), and leads to crystallization of the frog venom, the most potent venom known, in 1964 (15) (Table II). As the toxicity of these venoms goes up, their quantity goes down. Several hundred grams of crystalline starting material were available for structural work on the toad venoms and samandarin.

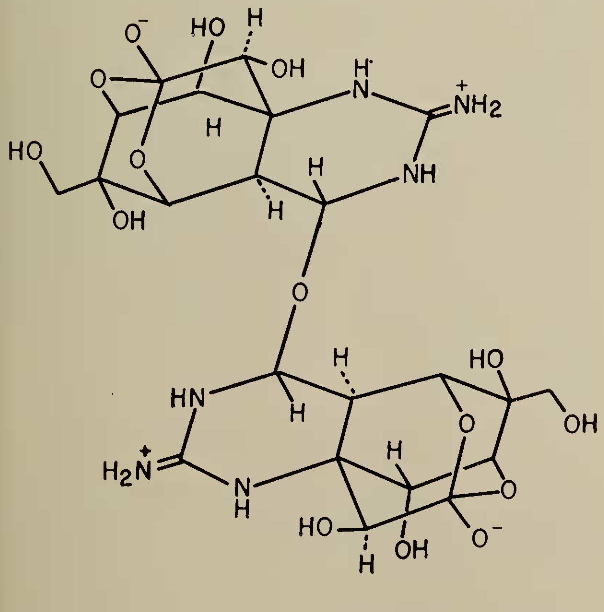
A comparison of the collection procedure is instructive: A ten-day collection in the environs of Freiburg (Black Forest) netted a quarry of 33,000 toads *(Bufo bufo bufo)* which were "milked" by placing under an inverted bowl and expressing the venom out of the parotid glands (located behind the eyes) with flat forceps. The stream of milky fluid is caught on the walls of the bowl and in cotton. The animal is set free at the place of capture with no injurious consequence. From 33,000 toads, 36 g. of crystalline bufotalin VI and 29 g. of companion venoms were obtained.

By contrast, the first expedition into the Choco jungle of Western Colombia (annual rainfall over 11 yards), under the courageous leadership of Mrs. Martè Latham, within 8 weeks yielded only 330 of the tiny and elusive poison arrow frogs, whose capture is infinitely more difficult than that of the clumsy and heavy European toad. Our Indian helpers used a little trick: they skillfully imitated the frog's peeping which sounds like fiú-fiú-fiú, by whistling and at the same time tapping

Table II. Comparative Tabulation of Venoms from Amphibians: Toads, Salamanders, and Frogs

Amphibian	Average Weight of Single Animal	Amount of Venom per Animal	Individual Components of Venom
Bufo alvarius (North America)	284 g	0.44 g	Bufotalin Bufotalinin
Bufo marinus (South America)	230 g	0.58 g	Marinobufagin Telocinobufagin
Bufo bufo bufo (Europe)	27 g	0.016-0.027 g	Bufotoxin
Salamandra maculosa taeniata (Fire salamander, Belgium, Spain)	14-18 g	0.042 g	Samandarine Samandarone Samandaridine Cycloneosamandione
Salamandra maculosa maculosa (Balcan subspecies)	18-24 g	0.05 g	O-Acetylsamandarine Samandarone Samandaridine Cycloneosamandione
Salamandra atra (Alpine salamander, Tyrol)	6.2 g	0.032-0.035 g	Samandarine Samandarone Samandaridine
Phyllobates bicolor (Poison arrow frog of Western Colombia)	1 g	0.001 g	Batrachotoxin Batrachotoxinin A Batrachotoxinin B Batrachotoxinin C

their cheek with their fingers. Their imitation is so perfect that a frog not too far away usually answers the call and thus can be located. Trying to find these small frogs which live well-hidden under the tropical ground cover, by any other means, would seem hopeless.



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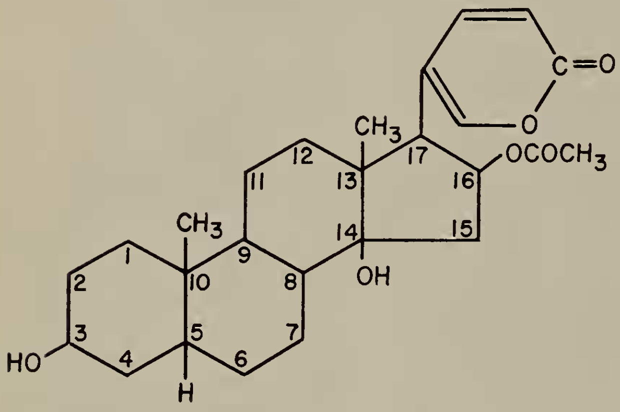
The kokoi frog, as the Cholo Indians call it, is 2-3 cm. long and averages only one gram in weight. Frogs have no parotid glands and the venom is located in the skins, from which it is extracted by aqueous methanol. The skin is black, with either two small yellow stripes along the back or two broad bands of a deep reddish yellow, with dots of the same color sprinkled in between these bands. This bicolorism reminds one of the similar but much stronger black-yellow skin pattern of the fire salamander, where the yellow color, a warning signal to other animals, consists of riboflavin which may be either bound to protein, or form an occlusion complex with guanin in the guanophorous cells of the epiderm (16).

Salamanders (15-18 g.) contain up to 40 mg. of crystalline alkaloids. If one extrapolates these figures to human conditions, a man of 80 kg. body weight would carry in his skin 150-180 g. of samandarin and congeners, *i.e.*, a poison with one-third the toxicity of strychnine. Although normally salamanders make no active use of their venom, they may force the venom out of their skin glands at the last extremity.

Although there was no dearth of salamander alkaloids and a wealth of chemical information, the interesting and novel steroidal systems of samandarine (VII) and cycloneosamandione (VIII) had to be established by roentgenographic analysis.



VI. BUFOTALIN

Like bufotalin (VI), samandarin (VII) has an oxygen function in the C_{16}-position, and in the venom of *Salamandra maculosa maculosa* this hydroxyl is also acetylated (17). The related ketone, samandarone, shows a rotatory dispersion curve with a negative Cotton effect, whose interpretation leads to the relative and absolute configurational assignments as expressed in VII. Cycloneosamandione (VIII) contains the unusual α-aldehyde group at C-10, which becomes free on reaction with methyl iodide to form N-methyl-neosamanonol methiodide (IX) whose Cotton effect is opposite to that of carotoxigenin (5α, 10β); VIII is the first natural steroid with the anomalous α-C-10 configuration (18).

The empirical formula of batrachotoxin was first established with 50 micrograms of amorphous material. The advent of the double-focusing mass spectrophotometer made possible this more-than-hundredfold increase in analytical sensitivity. Without this advance, structural elucidations on a microgram scale would not be possible. All chemical reactions were carried out with less than 50 γ of batrachotoxin. The products of these reactions were purified by thin-layer chromatography and then injected into the mass spectrometer. To judge from the available "cracking patterns" and the new method of "element mapping", batrachotoxin should possess a steroid-type carbon skeleton X, to one terminus of which (ring A or D) is attached the $C_4H_{8-10}NO$ grouping which in turn should have another oxygen atom within three additional carbon atoms. Although the steroidal skeleton is common to the venoms of sea cucumber, toad, salamander, and kokoi frog, there are unique and novel chemical features in each structure. Batrachotoxin does not have the unusual 3-aza-A-homo-5β-androstan structure of samandarin. Its most unusual feature is the weakly basic nitrogen and its particular environment which are currently the subject of detailed investigation on the microgram level. In that respect a new dimension has been added to the structural elucidation of natural products (19).

However, structural elucidation *per se* is no longer a primary aim, but only a prerequisite for entering into the dynamic aspects of cell components. Poisonous animals have given us the first clues on the occurrence, biosynthesis, and interrelationships of endogenous amines, such as serotonin, octopamine etc., which were later discovered in human metabolism. Conversely, enzymes involved in the biosynthesis and breakdown of catechol- and indolealkyl-amines in mammalian organisms have later been located and identified in the toad (20).

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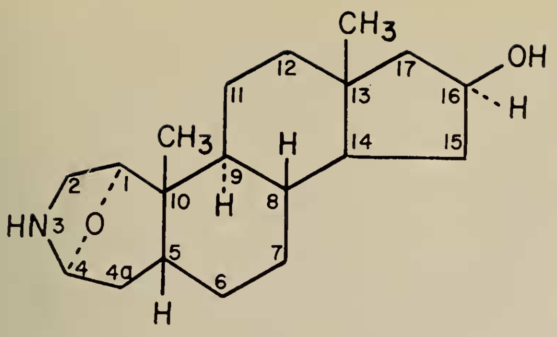
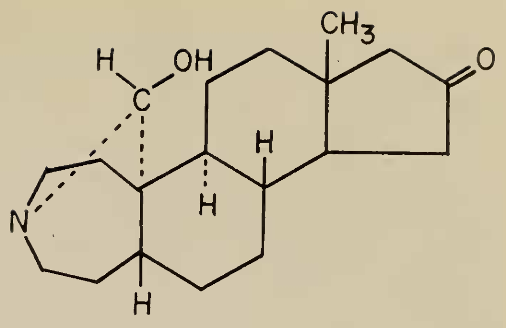
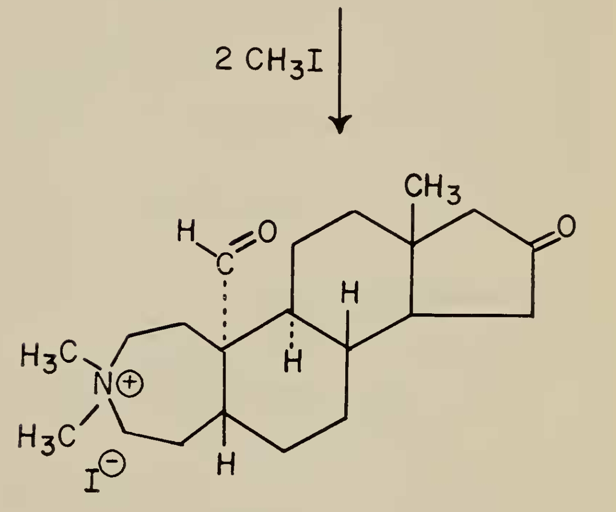


VII. SAMANDARINE



VIII. CYCLONEOSAMANDIONE



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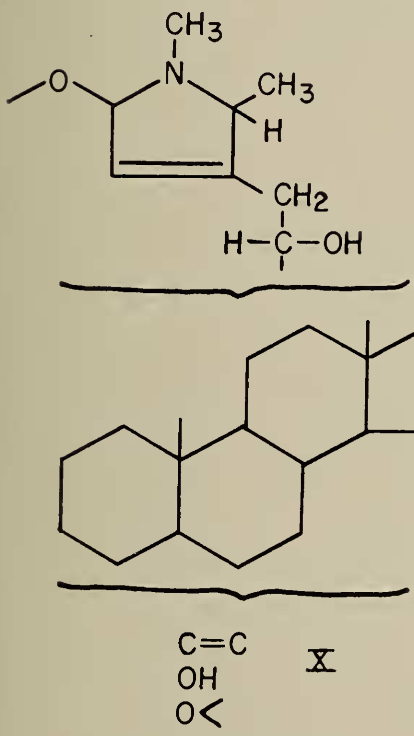

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A CONTRIBUTION FROM THE ARCHIVIST

A Forester's Thoughts in the Journal of 1915

Forestry, to which the February issue of the Journal was dedicated, was discussed in a previous issue 50 years ago, in a long article, "The Place of Forestry Among the Natural Sciences" (Journal 5, 41-57 (1915)). It was the text of an address delivered before the Academy on December 3, 1914, by Henry Solon Graves (May 3, 1871-March 7, 1951), chief of the U. S. Forest Service from 1910 to 1920. His main objective was to define forestry as "tree sociology" into which anatomy and physiology enter "only as one of the essential parts without which it is impossible to grasp the processes that take place in the forest." He used this opportunity to mention proudly that the Forest Service "is now spending nearly $300,000 annually for research work." In the Annual Report of the Department of Agriculture for the year ended June 30, 1915, Graves gave some results of this research (page 187), which led to improvements in hardwood distillation, turpentining, and the utilization of sawdust by hydrolysis and subsequent alcoholic fermentation.

Graves was a lieutenant colonel in the Corps of Engineers, 1917-19, and Sterling professor of forestry at Yale, 1922-39. In 1947 he was decorated by the French government with the Cross of Officier du Mérite Agricole. His book on forest mensuration first appeared in 1906.

Here is the heart of his story from the 1915 Journal, pages 44-5:

> Forestry as a natural science, therefore, deals with the forest as a community in which the individual trees influence one another and also influence the character and life of the community itself. As a community the forest has individual character and form. It has a definite life history; it grows, develops, matures, and propagates itself. Its form, development and final total product may be modified by external influences. By abuse it may be greatly injured and the forest as a living entity may even be destroyed. It responds equally to care and may be so molded by skillful treatment as to produce a high quality of product, and in greater amount and in a shorter time than if left to nature. The life history of this forest community varies according to the species composing it, the density of the stand, the manner in which the trees of different ages are grouped, the climatic and soil factors which affect the vigor and growth of the individual trees. The simplest form of a forest community is that composed of trees of one species and all of the same age. When several species and trees of different ages occupy the same ground, the form is more complex, the crowns overlapping and the roots occupying different layers of the soil. Thus, for instance, when the ground is occupied with a mixed stand of Douglas fir and hemlock, the former requiring more light, occupies the upper story, and because of its deeper root system extends to the lower lying strata of the soil. The hemlock, on the other hand, which is capable of growing under shade, occupies the under story, and having shallow roots utilizes largely the top soil.
>
> These are forest communities, such for instance as those typical of northwestern Idaho, where western larch, Douglas fir, western white pine, white fir, western red cedar, and hemlock all grow together. Such a forest is evidently a very complex organism, the stability of which is based on a very nice adjustment between the different classes and groups occupying the same ground. Any change in one of these classes or groups must necessarily affect the other. If, for instance, in the Douglas fir-hemlock forest, the Douglas fir is cut out, the remaining hemlock trees are likely to die out because their shallow roots are left exposed to the drying effect of the sun and wind. It is only by a thorough understanding of